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Mechanized Toxicological Serum Tests in Screening Hospitalized Patients

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Summary: A spectrum of quantitative and qualitative methods was adapted to the RA-1000/RA-XT selective analyser for the purpose of excluding or detecting common types of intoxication in the emergency laboratory of our primary care community hospital. Ethanol and salicylates (measured photometrically) and acetaminophen (measured immunologically by EMIT tox) were quantitatively analysed in serum. Immunological group tests (EMIT tox) for barbiturates, benzodiazepines, tricyclic antidepressants and related compounds were used for qualitative analysis. Well established clinical chemical methods (aspartarte aminotransferase, alanine aminotransferase, creatine kinase, pseudocholinesterase, glucose and lactate) were applied to the serum samples using the same selective analyser. Within and between run precision, accuracy, recovery and detection ranges (linearity) fulfilled the recommendations of forefield toxicological analysis for all methods.

Ethanol (g/l), measured photometrically with the RA-1000 analyser, agreed with the reference method (headspace gas-chromatography) with a correlation coefficient > 0.99 ($y = 0.06 + 0.98x$). Acetaminophen and salicylates showed correlation coefficients > 0.94 and > 0.99, when compared with manual colorimetric procedures (acetaminophen (mg/l): $y = -3.22 + 0.896x$; salicylates (mg/l): $y = -2.1 + 1x$). Qualitative group tests for barbiturates, benzodiazepines and tricyclic antidepressants measured with the RA-1000 analyser were in good agreement with the EMIT single test procedure.

The ranges of the quantitative methods allowed quantification of analytes from therapeutic (non-toxic) to very high levels in undiluted samples (ethanol 0.05 up to 4 g/l; salicylates 32 up to 1200 mg/l and acetaminophen 1.9 up to 200 mg/l). The low detection limits of the qualitative tests allowed the recognition of compounds in plasma that were present in low concentrations and/or displayed only minor reactivity with the antibodies provided by the EMIT tox test kits.

As a consequence, decision limits for all three group tests in serum were lowered to near the detection limit:

Barbiturates:	0.5 mg/l (calibrated with secobarbital)
Benzodiazepines:	0.05 mg/l (calibrated with nordiazepam)
Tricyclic antidepressants:	0.07 mg/l (calibrated with nortriptyline)

For quantitative tests the lower limits of quantification were:

Acetaminophen:	10 mg/l;
Salicylates:	50 mg/l;
Ethanol:	0.15 g/l.

The working reagents were stable for at least 14 days at 4–8 °C. Calibration curves were stable over the expiration period of reconstituted original reagents (6–12 weeks), also when working reagents were prepared in aliquots from stored reconstituted reagents.

Application of the newly adapted programme to serum samples of nearly two hundred patients showed it to be suitable for screening patients in which intoxication is suspected or needs to be excluded.

Introduction

Despite the importance of checking for acute intoxication and chronic drug abuse in newly hospitalized patients, toxicological analysis is not practised as widely in German hospitals (1) as recommended by an expert group (2). This is probably due to the effort required for the application of most of the available procedures.

Intoxications are usually first detected by qualitative tests (3) based either on direct examination of urine or of acidic or basic extracts. Moreover, drug-abundance control programmes are commonly based on the analysis of urine (4), because most substances can be detected for a longer period in urine than in serum. However, the severity of an intoxication can only be derived from plasma values. Apparently, the procedures based on urine analysis (5) do not adequately meet the requirements for detection or exclusion of acute intoxication in the emergency laboratory. Serum or plasma is the primary sample material of newly hospitalized patients, and its drug content mirrors the actual state of intoxication better than that of urine. An analytical programme for serum or plasma therefore seemed more appropriate.

Many substances appear in only low levels in plasma and urine, following the administration of toxicologically relevant doses. Furthermore, various immunological detection systems used for group-specific measurements differ widely in their sensitivity to different substances within the one group. To overcome these problems, extraction, hydrolysis of conjugates and reduction of decision limits have been suggested as appropriate solutions (6–9). For the present needs of a toxicological emergency programme only those procedures which need little additional effort seemed appropriate.

Our purpose was therefore to develop an automated toxicological screening programme for serum by combining quantitative and qualitative measurements, and to considerably reduce the operational time and the cost by adapting the methodology to a selective analyser.

The aim was to exclude, differentiate and quantify various frequent forms of intoxication. The present methodology can be adapted to a great number of other mechanised analysers. Parts of this work have been presented in a preliminary form (10–12).

Materials and Methods

Plasma or serum samples

Samples were drawn as soon as possible after admission of the patient to the hospital. Blood was collected in 4 ml serum or plasma separator tubes (Vacutainer®, Becton Dickinson, Meylan Cedex, France) with lithium heparinate as anticoagulant.

Analytical procedures

Table 1 summarizes the parameters for adaptation of qualitative procedures, table 2 of quantitative procedures to the RA-1000/RA-XT selective analyser (Bayer Diagnostik-Technicon, München, Germany). A software version including immunoassay test capabilities is recommended.

Barbiturates

Barbiturates were measured by enzyme multiplied immunoassay (EMIT), using a test kit (EMIT tox barbiturates) purchased from Syva, Darmstadt, Germany. Stock reagents were prepared as prescribed by the manufacturer. For preparation of working solutions, reagent 1 (group-specific antibodies, substrates) and reagent 2 (enzyme-linked secobarbital as tracer) were diluted 10-fold with the buffer solution. Using the RA-1000 analyser, plasma (3.5 µl) was mixed with 175 µl working solution 1, and 30 s later 175 µl working solution 2 was added. After an additional 30 s the kinetics were recorded in a period of 30 s. Barbiturate calibrators A–C (Abbott Diagnostics, Wiesbaden, Germany) were used. A calibration curve was constructed using these standards or mixtures thereof containing concentrations of 0, 0.5, 2.0 and 3.5 mg/l secobarbital respectively.

This method was compared with the EMIT single test (Syva, Darmstadt, Germany), using the photometer from the same manufacturer.

Benzodiazepines

The procedure for benzodiazepines was analogous to the method for barbiturates using a test kit (EMIT tox benzodiazepines) from Syva, Darmstadt, Germany.

Benzodiazepine calibrators A–F (Abbott Diagnostics, Wiesbaden, Germany) were used as standards. A calibration curve was constructed using concentrations of 0, 0.05, 0.1, 0.2 and 0.4 mg/l nordiazepam.

This method was compared with the EMIT single test (Syva, Darmstadt, Germany).

Tricyclic antidepressants

Tricyclic antidepressants and related compounds were also measured by adaptation of an EMIT procedure (EMIT tox TCA, Syva, Darmstadt, Germany) to the RA-1000 analyser.

Tricyclic Antidepressant calibrators A–D (Abbott Diagnostics, Wiesbaden, Germany) were used as standards. A calibration curve was constructed using concentrations of 0, 0.07, 0.15 and 0.3 mg/l nortriptyline.

This method was compared with the EMIT single test (Syva, Darmstadt, Germany).

Tab. 1. Instrumental settings for qualitative EMIT tests in plasma on the RA-1000 analyser.

Parameter (group reaction)	Barbiturates	Benzodiazepines	Tricyclic antidepressants
Reaction type*	1	1	1
Sample volume	7% (3.5 µl)	7% (3.5 µl)	7% (3.5 µl)
Wavelength	1 (340 nm)	1 (340 nm)	1 (340 nm)
Preincubation time**	60 s	60 s	60 s
Incubation time	30 s	30 s	30 s
Reading time	30 s	30 s	30 s
Reagent 1 volume	35% (175 µl)	35% (175 µl)	35% (175 µl)
Reagent 2 volume	35% (175 µl)	35% (175 µl)	35% (175 µl)
Unit	7 (mg/l)	7 (mg/l)	7 (mg/l)
Detection limit***	5.0	0.6	0.5
Normal range**** +	0.5	0.05	0.07
1.0. limit	0.05	0.05	0.05
Immunoassay type	0	0	0
Calibrator 1	0	0	0
Calibrator 2	0.5	0.05	0.07
Calibrator 3	2.0	0.1	0.15
Calibrator 4	3.5	0.2	0.3
Calibrator 5	–	0.4	–
LIM 1 to LIM 4 (5)	10	10	10
SLM 1 to SLM 4 (5)	99	99	99
10/50/90 PAW	99.00	99.00	99.00
%10/%50/%100 PAW	99	99	99
SFQ LIMIT	10	10	10

* 1 = 2 point kinetics
** General setting for a reagent tray
*** not equivalent to diluting limit
**** equivalent to the decision limit
LIM = maximal allowable CV (%) for replicate aspirations of corresponding standard
SLM = normalized deviation = (fitted value − expected value) value for maximal standard concentration
PAW = predicted concentration when the assay value for a standard is multiplied by the indicated percentage, and then applied to the standard curve
%PAW = maximal allowable deviation (in) from the predicted value specified by the PAW parameter
SFQ = limit on residual sum of squares of fitted calibration curve

Tab. 2. Instrumental settings for quantitative determination of the plasma analytes on the RA-1000 analyser.

Parameter	Acetaminophen	Salicylates	Ethanol
Reaction type*	1	2	2
Sample volume	7% (3.5 µl)	60% (30 µl)	4% (2 µl)
Wavelength	1 (340 nm)	5 (550 nm)	1 (340 nm)
Preincubation time**	60 s	60 s	–
Incubation time	30 s	300 s	300 s
Reading time	30 s	–	–
Reagent 1 volume	35% (175 µl)	67% (335 µl)	75% (375 µl)
Reagent 2 volume	35% (175 µl)	7% (35 µl)	–
Unit	7 (mg/l)	7 (mg/l)	(g/l)
Detection limit	200 mg/l	1200 mg/l	4 g/l
Cal. Factor	–	xxxx	1.99
Normal range +***	10 mg/l	50 mg/l	0.15 g/l
Reagent blank	–	0.0	0.0
1.0. limit	1.0	–	–
Endpoint lim.	–	0.02	0.05
Immunoassay type	0	–	–
Calibrator 1	0 mg/l	250 mg/l	–
Calibrator 2	10 mg/l	–	–
Calibrator 3	25 mg/l	–	–
Calibrator 4	50 mg/l	–	–
Calibrator 5	100 mg/l	–	–
Calibrator 6	200 mg/l	–	–
LIM 1 to LIM 6	10	–	–
SLM 1 to SLM 6	99	–	–
10/50/90 PAW	99.0	–	–
%10/%50/%90 PAW	99	–	–
SFQ LIM	10	–	–

* 1 = 2 point kinetics; 2 = endpoint ** General setting for a reagent tray *** equivalent to the decision limit

Acetaminophen

The EMIT procedure (EMIT tox Acetaminophen, Syva, Darmstadt) was adapted to the RA-1000 analyser using working reagents prepared as for the qualitative methods above.

For preparation of the standard curve, calibrators provided with the test kit were used. The calibration curve was constructed using concentrations of 0, 10, 25, 50, 100 and 200 mg/l.

This method was compared with a manual colorimetric method after enzymatic hydrolysis (13) using a test kit purchased from Cambridge Life Sciences, London, UK.

Salicylates

A colorimetric procedure using a test kit from Sigma, Deisenhofen, Germany was adapted to the RA-1000 selective analyser. The blank reagent provided was used as working solution 1. Working solution 2 was prepared by mixing 7.6 parts blank reagent and one part colour reagent, and this mixture was stable at 4 °C for at least 4 weeks. As can be seen from table 2, 30 µl plasma were mixed with 335 µl working solution 1 and 60 seconds later 35 µl working solution 2 was added. After 300 s the final reading at 550 nm was taken. A standard solution of 250 mg/l salicylate provided with the test kit was used for calibration. A sample blank using working reagent 1 alone was measured in parallel.

This method was compared with the manual method using the same test kit from Sigma.

Ethanol

The alcohol dehydrogenase (EC 1.1.1.1) method was adapted to the RA-1000 analyser. Reagents, i. e. NAD/ADH-suspension and glycine buffer containing a non-specified aldehyde captor, were purchased from Sigma (Deisenhofen, Germany). Working reagent was prepared as prescribed by the manufacturer, and it was stable for at least 48 hours below 15 °C. Plasma (2 µl) was mixed with 375 µl reagent and a final reading at 340 nm was taken 300 s thereafter. The concentration of ethanol was calculated, using the molar absorbance coefficient of NADH at 340 nm and the factor of 1.992. The method was compared with the headspace gas chromatography procedure according to l. c. (14).

Control materials

The intra-assay and inter-assay precision for the analysis of all analytes except ethanol were determined with a mixture of Lyphochek TDM Level-1 (Biorad, Munich, Germany) and Control Benzodiazepine Serum Level-M (Abbott Diagnostics, Wiesbaden, Germany). The mixture was prepared by dissolving the Lyphochek control in the fluid benzodiazepine control (2.5 ml), followed by the addition of 2.5 ml distilled water. Samples from patients were also used for assessing precision data.

To determine the precision and accuracy of the ethanol method, different charges of Fluinorm Ethanol R Level I and II (Behringwerke, Marburg, Germany) were used. The accuracy of the acetaminophen assay was measured with Lyphochek TDM Level-3 (Biorad, Munich, Germany) and Control Acetaminophen (Syva, Darmstadt, Germany). Lyphochek TDM Level 2/3 were used to determine the accuracy of the salicylate assay. The calibration curves of the qualitative determinations were checked with Barbiturates Control L, Benzodiazepines Control L and Tricyclic Antidepressants Control M (all from Abbott, Wiesbaden, Germany), and additionally with EMIT st Serum Calibrator and EMIT st tricyclic antidepressants Calibrator (Syva, Darmstadt, Germany).

Statistical evaluation

Linear regression analysis was performed using the least square analysis method (15). In addition, the general regression procedure described by *Bablok* et al. was applied using the computer program provided by these authors (16).

The analytical sensitivity of the qualitative immunological screening tests was evaluated by the method introduced by *Kutter* (17). The decision limit of the qualitative tests was extended as described in l. c. (8, 18, 19).

Test results for qualitative methods were compared by testing for discordancies with the four-field-technique.

Results

Adaptation of methods

The aim of the present study was to take a combination of quantitative and qualitative methods for recognizing acute intoxication with frequently abused drugs, and to adapt them to an analyser, which is used routinely in the clinical chemistry emergency laboratory. Using the settings summarized in table 1 and table 2 this was realised on the RA-1000/RAXT analyser. The total programme can be performed in 10 minutes using heparin plasma or serum samples.

In addition, bidirectional communication with a laboratory computer system reduces the work of the technician in delivering sample and control into the sample cups and in starting the analyser.

Detection and linearity limits

The detection limit of the assays (mean + 3 SD) was determined by measuring 30 different drug-free samples (tab. 3). The immunological assays showed a linear response up to the highest calibrator level, and this was confirmed by measuring different dilutions of samples exhibiting high drug concentrations.

Test results including qualitative determination exceeding the calibration range should be confirmed by analysing a diluted sample. It is recommended that dilution be performed with a drug-free sample; most control materials used in clinical chemistry are suitable for this purpose. As shown in table 3 the linearity limits for ethanol, acetaminophen and salicylates were 4.0 g/l, 200 mg/l and 1200 mg/l, respectively.

Analytical precision and accuracy

The intra-assay and inter-assay precisions are summarized in table 4.

Intra- and inter-assay precision for ethanol was in the usual range for alcohol dehydrogenase methods

Tab. 3. Detection and linearity limits of the qualitative and quantitative methods used.

Analyte	Unit	Detection limit	Linearity limit
Barbiturates	mg/l	0.04	3.5
Benzodiazepines	mg/l	0.04	0.4
Tricyclic antidepressants	mg/l	(0)	0.3
Acetaminophen	mg/l	1.9	200
Salicylates	mg/l	32	1200
Ethanol	g/l	0.04	4.0

(CV% < 8%)[1]). Variations for acetaminophen and salicylates were in the range usually accepted for drug monitoring, i. e. less than 10%.

Although barbiturates, benzodiazepines and tricyclic antidepressants are only determined qualitatively, precision data can be given, because primary results are quantitative and transformed into qualitative results by setting a decision limit.

A special feature of the EMIT principle is the sometimes higher precision with low rather than high analyte concentrations. Accuracies measured with control materials are summarized in table 5. Although the methods used for determination of the target values were not the same as the adapted methods, the deviations lay in an acceptable range for screening determinations.

Correlation with comparison methods

Serum determinations of barbiturates, benzodiazepines and tricyclic antidepressants were compared with results of the EMIT single test using the decision limit suggested by the manufacturer and a lowered decision limit. No negative results (fig. 1) were ob-

[1]) permitted limit of deviation for low levels of ethanol (external quality control from the Deutsche Gesellschaft für Klinische Chemie, Bonn, Germany)

Tab. 4. Analytical precision of automated procedures for qualitative and quantitative methods in serum.

Analyte	N	Within-run			N	Between-run		
		Mean	SD	CV (%)		Mean	SD	CV (%)
Barbiturates (mg/l)	10	0.80	0.04	11	19	1.09	0.38	35*
	10	1.88	0.13	7	6	3.17	0.24	8
Benzodiazepines (mg/l)	10	0.22	0.01	5	19	0.25	0.01	4
	10	0.27	0.02	8	6	0.30	0.05	17
Tricyclic antidepressants (mg/l)	10	0.23	0.009	4	19	0.23	0.02	9
	10	0.42	0.02	5	6	0.18	0.02	11
Acetaminophen (mg/l)	10	46.5	6.1	13	8	128	7.4	6
Salicylates (mg/l)	10	73.4	2.67	4	19	71.8	3.24	5
	10	157.1	1.5	1	6	133	0.8	1
Ethanol (g/l)	10	0.65	0.03	4.6	20	0.60	0.02	3
	10	2.75	0.034	1.2	10	2.03	0.045	2.2

* concentration near detection limit

Tab. 5. Accuracy of automated procedures for qualitative and quantitative methods in serum.

Analyte	Material	Target value mg/l	Result mg/l	Difference mg/l	Deviation %
Barbiturates	Abbott L	3.0	2.9	0.1	− 3
	EMIT st	3.0	2.83	0.17	− 6
Benzodiazepines	Abbott L	0.3	0.33	0.03	+10
	EMIT st	0.3	0.32	0.02	+ 7
Tricyclic antidepressants	Abbott M	0.2	0.22	0.02	+10
	EMIT st	0.2	0.16	0.04	−20
Acetaminophen	Syva Control	75	78.9	3.9	+ 5
	Biorad TDM 3	135	126	9	− 7
Salicylates	Biorad TDM 2	156	159	3	+ 2
	Biorad TDM 3	454	490	36	+ 8
Ethanol	Fluinorm 1	0.63	0.62	0.01	+ 1
	Fluinorm 2	1.52	1.55	0.03	+ 2

a) Barbiturates

		EMIT tox (RA-1000) +	EMIT tox (RA-1000) −
EMIT st proposed cut-off	+	7	0
	−	6	60
EMIT st lowered cut-off	+	10	3
	−	3	57

b) Benzodiazepines

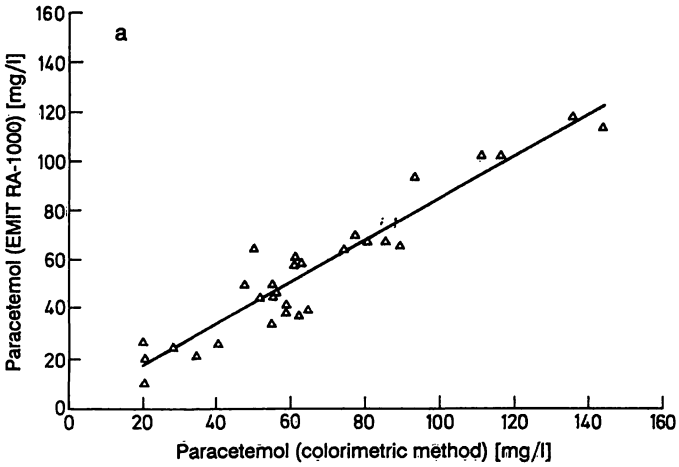
		EMIT tox (RA-1000) +	EMIT tox (RA-1000) −
EMIT st proposed cut-off	+	15	0
	−	9	49
EMIT st lowered cut-off	+	20	2
	−	4	47

c) Tricyclic antidepressants

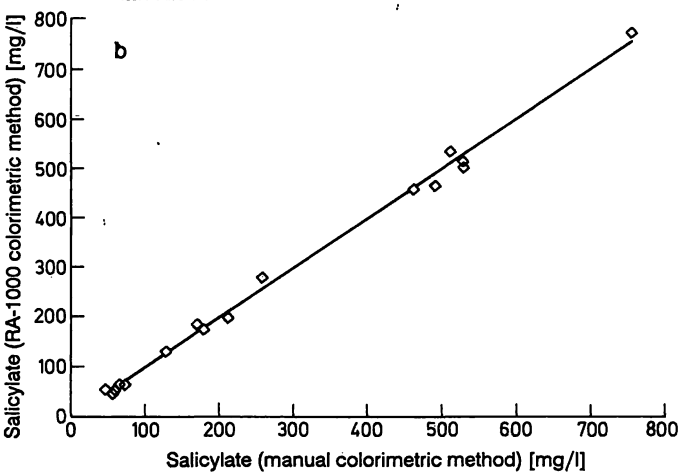
		EMIT tox (RA-1000) +	EMIT tox (RA-1000) −
EMIT st proposed cut-off	+	3	0
	−	3	46
EMIT st lowered cut-off	+	4	0
	−	2	46

Fig. 1. Discrepancies between determinations using the RA-1000 analyser and the EMIT st. For EMIT st two decision limits were used, that proposed by the manufacturer (3.0 mg/l secobarbital; 0.3 mg/l diazepam; 0.2 mg/l nortriptyline) and the detection limit (sample blank).

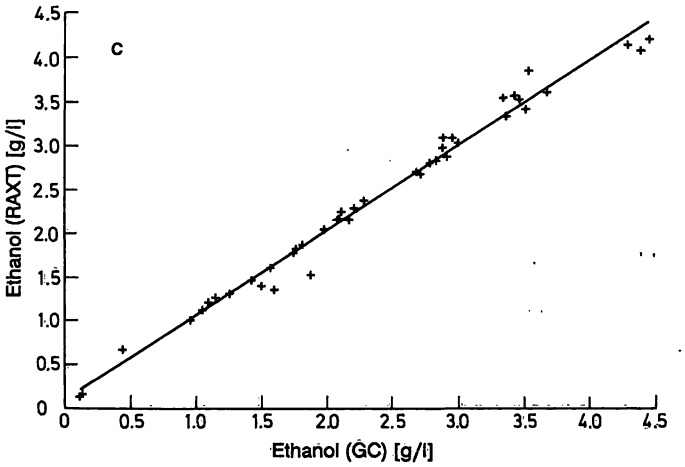
served with the automated method on the RA-1000 in comparison with the single test procedure. The greater number of positive results is probably due to the lower detection, e. g. decision limit of the automated procedure.



a. acetaminophen (paracetamol) compared with the method of *Price* (13).
linear regression: n = 30; r = 0.946
y = −0.12 + 0.85x
Bablok et al.: y = −3.22 + 0.896x
10 more samples gave results < 10 mg/l with both methods.



b. salicylate compared with the manual colorimetric method.
linear regression: n = 16; r = 0.998
y = −1.5 + 0.998x
Bablok et al.: y = −2.1 + 1x
10 more samples gave results < 50 mg/l with both methods.



c. ethanol compared with headspace gas chromatography.
linear regression: n = 42; r = 0.993
y = 0.09 + 0.97x
Bablok et al.: y = 0.06 + 0.98x
16 more samples gave results < 0.15 g/l with both methods.

Fig. 2. Comparison of results from the present quantitative analytical procedures with those from other routinely used methods.
16–42 samples were analysed with the present quantitative procedures and with the comparison methods as indicated. Linear regression was calculated by the method of least squares (15) and by the method of *Bablok* et al. (16).

The EMIT method for acetaminophen, which was adapted to the RA-1000 analyser, was compared with the manual colorimetric method of *Price* (13). The correlation coefficient was 0.946 (fig. 2a). From figure 2a deviations up to 50% from the regression line for low concentrations can be seen. However, both methods fulfil the clinical requirements for detection and quantification of acetaminophen overdosage.

Figure 2b compares the results for the analysis of salicylates in serum, using the present method and a manual procedure with the same test kit.

The ethanol determinations on the RA-1000/RAXT were compared with the candidate reference method (headspace gas chromatography). The results in figure 2c exhibited a good correlation (r = 0.993). Only a very small intercept due to the more extended detection limit of gas chromatography was detected.

Stability of working reagents and calibration curves

Reconstituted reagents for EMIT methods and salicylates exhibited stability for at least 6 weeks at 4—8 °C. In routine use over three years, the stability of prediluted working reagents at 4—8 °C was generally at least 14 days, as seen from a typical stability curve of controls measured daily without any recalibration (fig. 3).

The validity of calibration curves, when using working reagents from the same test kit, was checked by measuring accuracy controls. The maximal deviation allowed for keeping the old calibration curve was 30% for the qualitative procedures. It was usually unnecessary to perform a new calibration, when working solutions were prepared from the same reagent bottle.

Ethanol reagents were freshly prepared every two days, because of their limited stability.

The stability of serum samples using Vacutainer® tubes has been reported by others (20).

Analytical sensitivity and setting of the decision limits

The detection limits (sample blank) of the qualitative assays were found to be very low (tab. 3). The zero calibrator exhibited a higher absorption signal than nearly all the drug-free samples that were tested. This indicated a matrix difference between calibrators and freshly sampled serum or plasma, resulting in an obviously very low matrix dependence. To avoid possibly positive results from drug traces we decided to set the decision limits to the first calibrator concentration above zero.

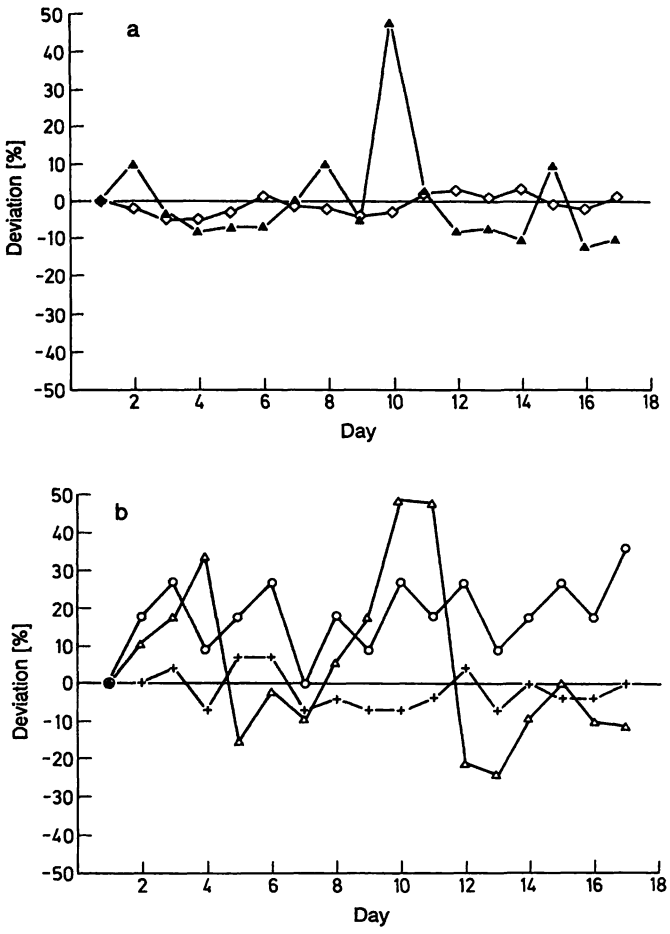


Fig. 3. Stability of reagents and calibration curves. Samples of a precision control material were analysed once a day over a period of 17 days without any recalibration. Values are given as deviation (%) from the measurement at the first day.
Reagents were stored at 4—8 °C. First values were: 105 mg/l salicylate (◇); 1.60 mg/l barbiturates (△); 0.11 mg/l benzodiazepines (○); 7.3 mg/l paracetamol (▲); 0.27 mg/l tricyclic antidepressants(+).
a. quantitative assays
b. qualitative assays

These decision limits were:

barbiturates:	0.5 mg/l (decision calibrator: secobarbital)
benzodiazepines:	0.05 mg/l (decision calibrator: nordiazepam)
tricyclic antidepressants:	0.07 mg/l (decision calibrator: nortriptyline)

Testing the analytical sensitivity for the determination of tricyclic antidepressants with the method introduced by *Kutter* (17), using a decision calibrator of 0.07 mg/l nortriptyline, resulted in N_{10} = 0.05 mg/l and N_{90} = 0.1 mg/l (fig. 4).

Lowering the decision limits produced a distinct increase of analytical sensitivity (tab. 6) compared with decision limits proposed by the manufacturer.

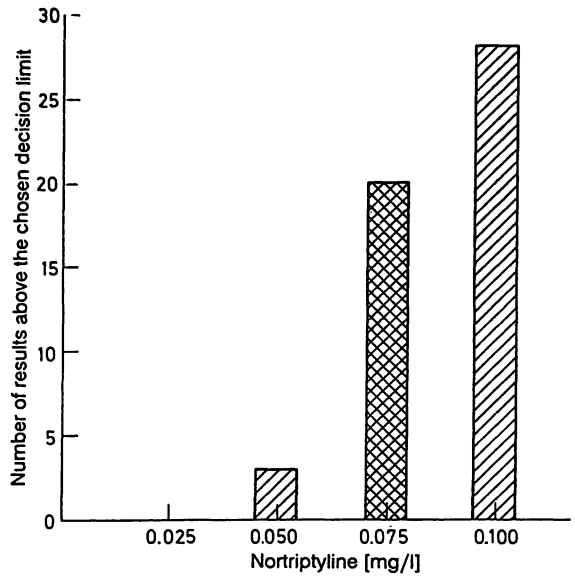


Fig. 4. Analytical sensitivity of the determination of tricyclic antidepressants.
According to *Kutter* (17), 30 different drug-free samples were spiked with increasing amount of nortriptyline and the concentrations measured were compared with the chosen decision limit (0.07 mg/l).

The decision values for the analgesics, paracetamol and salicylates, were set near to the upper therapeutic range limit (21):

acetaminophen: 10 mg/l
salicylates: 50 mg/l

Ethanol values less than 0.15 g/l were not given quantitatively, in accordance with the recommendations of an expert group (22).

Use of the new analytical programme as a screening procedure

In order to demonstrate the diagnostic utility of the present programme, the results of its use over a 6 month period in our laboratory are summarized in table 7.

When analysed with the present analytical programme, 139 out of 188 serum samples (74%) gave at least one positive result. As can be seen, benzodiazepines (40%) and ethanol (36%) were most frequently positive. The distribution of values observed is given in figure 5. Out of 188 samples, 28 (15%) were positive for benzodiazepines in combination with ethanol. However, other combinations were detected as well.

Only in 70 of these 188 cases (37%) were further determinations requested. Of these, 23 (33%) showed no evidence of intoxication in a more extended screening procedure, using various urine tests (12). Of the remaining 47 patients 15 gave a positive result for opiates in urine. A wide spectrum of other substances was detected with unspecific colour reactions, e. g. the tetrabromophenolphthaleinethylester reaction (23) and the *Forrest* reaction (3), therapeutic drug monitoring, thin layer chromatography and other immunoassays in urine. For clinical purposes, it was necessary to send samples from only 5 patients to external laboratories for confirmation and identification of the drugs involved.

Tab. 6. Effect of lowered decision limits.

	Barbiturates	Benzodiazepines	Tricyclic antidepressants
Proposed decision limit (mg/l)	3.0	0.3	0.2
Positive results	7	13	3
Negative results	69	63	52
Lowered decision limit (mg/l)	0.6	0.05	0.07
Positive results	15	23	6
Negative results	61	53	49

Tab. 7. Application of the analytical programme to patients, where intoxication was suspected from anamnesis or clinical examination.

a) Total results (with use of the lowered decision limits)

n = 188	Positive	Negative
Barbiturates	25	163
Benzodiazepines	81	107
Tricyclic antidepressants	27	161
Paracetamol	6	182
Salicylates	8	180
Ethanol	68	120

b) Combinations with positive benzodiazepines

n = 81	
Barbiturates	15
Tricyclic antidepressants	13
Paracetamol	3
Salicylates	5
Ethanol	28

c) Combinations with positive ethanol

n = 68	
Barbiturates	6
Benzodiazepines	28
Tricyclic antidepressants	10
Paracetamol	3
Salicylates	2

Discussion

General toxicological screening requires many analytical tools, e. g. colour tests (24), immunoassays (25), thin-layer chromatography (26, 27), gas chromatography (28–30), high-performance liquid chromato-

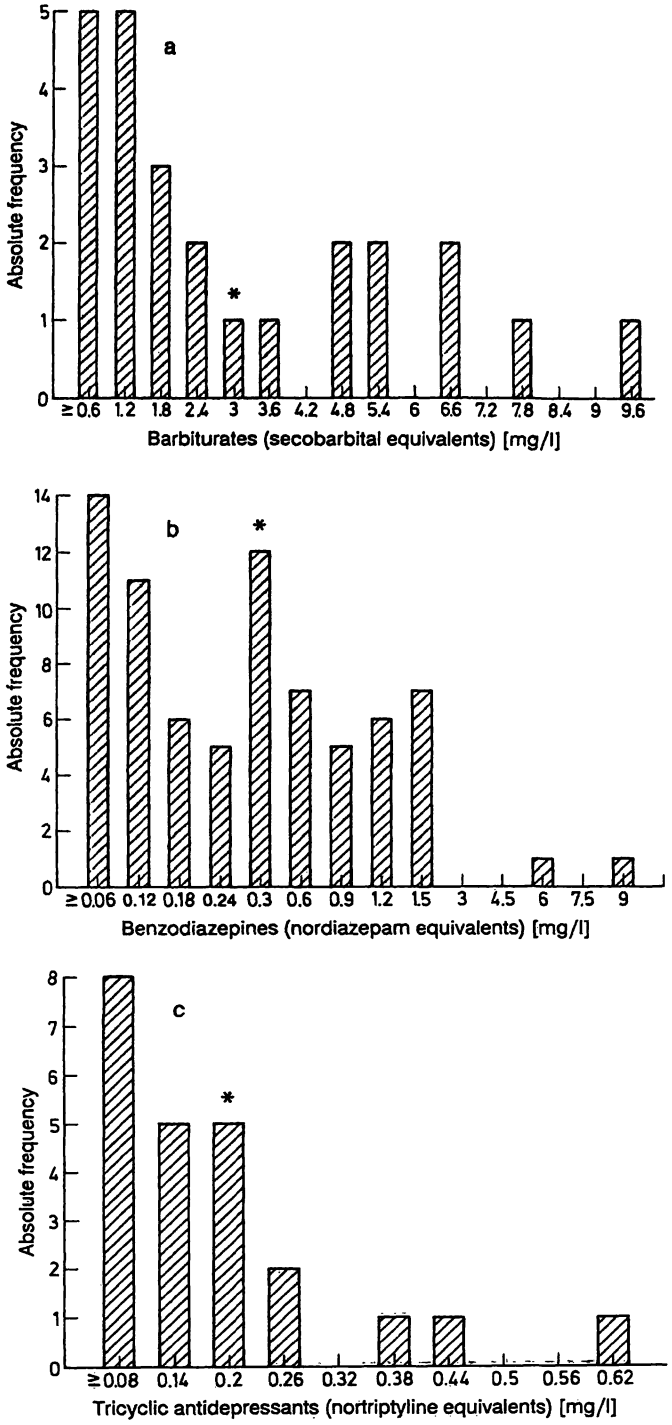


Fig. 5. Distribution of positive results measured in a total of 188 samples. The lowest concentration value given was used as the decision limit. The cut-off proposed by the manufacturer is marked by an asterisk.
a. barbiturate assay
b. benzodiazepine assay
c. tricyclic antidepressants assay

graphy (31, 32) and/or gas chromatography/mass-spectrometry (33, 34). In clinical toxicology, however, information obtained quickly with simple screening tests is very valuable (35–37). On the other hand, haematological, haemostaseological and clinical chemistry parameters are additionally useful in acute poisoning (36, 38). This prompted us to adapt assays for frequent toxicologically relevant compounds to an mechanized analyser, which is routinely used for emergency clinical chemistry. The resulting advantages of this strategy are: no additional technical or personnel requirements, no time-consuming procedures (for example extraction), and high analytical precision and accuracy with a remarkable decrease of reagent costs.

Decision limits

A major aim of toxicological forefield analyses is to exclude intoxication. Cut-off values of qualitative screening tests should be lowered for this purpose as far as possible to avoid false negative findings, especially with substances exhibiting low cross-reactivity. As a consequence, however, these cut-off values are in the therapeutic range for several drugs. The qualitative EMIT tests presented here do not discriminate between acute intoxications and results due to therapeutic drug application. The applicability of the screening programme is, however, not restricted by this fact, if the main aim of forefield toxicological analysis is exclusion of intoxication with a high degree of certainty.

False positive findings, on the other hand, can be corrected by the results of more specific tests. Two cut-off limits (for example the lower decision limit and that proposed by the producer) may be used to separate "clinically precarious positive" from positive results. However, this procedure does not eliminate the problems resulting from differences in cross-reactivity. According to a common agreement (39), each positive result implying therapeutic or other consequences should be confirmed by a separate test procedure.

Very low detection limits were estimated from measurements of thirty different drug-free serum samples for the EMIT serum tests. The obviously very small matrix dependence of these procedures, however, was not observed when the tests were calibrated with aqueous standards (data not shown).

In view of matrix dependency, the accuracy of the test procedures should be demonstrated with various control materials. These are quantitative tests, although they are only qualitatively interpreted. A quality assurance programme as used for quantitative measurements was therefore installed.

The decision limits for the quantitative analgesic determinations (paracetamol and salicylates) were set to the upper therapeutic range, in accordance with the common use of these drugs and the relatively great imprecision of the quantitative methods in the lower concentration range.

Application of the present programme as a screening procedure

The relative frequency of positive results with the different tests detected by our methodology was similar to the distribution of results found by *Gibitz* (36). These distributions are typical for non-specialized hospitals where intoxications of patients examined are usually not severe. Even in these cases, however, toxicological analysis is necessary for differential diagnostic purposes. Depending on the cut-off values used as the decision point for a positive result, the numbers of positives may vary by a factor of 2. As can be deduced from the distribution of positive results as a function of present concentration ranges (fig. 5), the setting of cut-off values as proposed by the producer seems not to be optimal, since nearly equal numbers of samples exhibited values that were higher and lower than these cut-off values in all three group tests. The setting of the decision limit near the detection limit (sample blank) therefore seems to be more reliable. When the detection limit (sample blank) was used as the decision limit, the positive and negative results agreed well with those found in the single test procedures. Also, for example, low positive signals in the test for tricyclic antidepressants may be due to diphenhydramine cross-reativity, e. g. unspecific reactivity, but this is desirable for forefield toxicology. To increase the number of intoxications detectable by the present programme it seems advisable to add further tests like an immunoassay for opiates (in urine), the tetrabromophenolphthaleinethylester reaction for the detection of basic compounds (23) and the *Forrest* reactions for the detection of phenothiazines (3).

Future aspects and conclusions

With respect to the necessity of automation in the modern emergency laboratory, we adapted toxicologically relevant test procedures for frequent drugs to the RA-1000/RAXT analyser. This programme might be extended in future by the inclusion of other immunoassays for more substances and/or groups, like diphenhydramin, antiarrhythmic and antihypertonic drugs. In our opinion the complete strategy for exclusion of intoxication will comprise the present fully automated programme, probably with some additions, and unspecific colour reactions. Positive results should then be further investigated and, if necessary, differentiated with more powerful technology such as gas chromatography/mass spectrometry.

In conclusion, the present serum screening programme proved to be a suitable first step in testing patients entering a non-specialized hospital, detecting 75% of total "positive" patients (11). The fully mechanized serum tests can be included in the programme of emergency laboratories, and they can be performed by the technician within 15 minutes with little addition to the workload. In addition the low reagent consumption keeps the reagent costs within an acceptable range.

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